

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
23 September 2004 (23.09.2004)

PCT

(10) International Publication Number
WO 2004/081027 A2

(51) International Patent Classification⁷: **C07K**
(21) International Application Number:
PCT/IB2004/001186
(22) International Filing Date: 15 March 2004 (15.03.2004)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data:
103 11 248.0 14 March 2003 (14.03.2003) DE

(71) Applicant (for all designated States except US): **ON-COMAB GMBH** [DE/DE]; Friedrich-Bergius-Ring 15, 97076 Würzburg (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MUELLER-HERMELINK, Hans-Konrad** [DE/DE]; Heinrich-Zeuner-Strasse 72, 97082 Würzburg (DE). **VOLLMERS, Heinz, Peter** [DE/DE]; Budapeststrasse 23, 97084 Würzburg (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **NEOPLASM SPECIFIC ANTIBODIES AND USES THEREOF**

(57) Abstract: The present invention features polypeptides, such as antibodies, and their use in the treatment and diagnosis of neoplasms.



WO 2004/081027 A2

BEST AVAILABLE COPY

NEOPLASM SPECIFIC ANTIBODIES AND USES THEREOF

5

Background of the Invention

The present invention is related to the field of cancer diagnosis and treatment and, more specifically, to the identification of polypeptides, such as antibodies, useful in the diagnosis, detection, monitoring, and treatment of neoplasms in a mammal, e.g.,
10 a human.

In the United States well over one million individuals are diagnosed with cancer each year. Although recent advances in the medical field have significantly improved the rate of survival among cancer patients, a large number of cancer-related deaths still could be prevented by the early diagnosis of the tumor. Accordingly, at
15 the time of initial diagnosis, an alarming number of patients have already reached late stages of the disease.

With respect to colorectal cancer, the prognosis is usually poor in 50% of all cases because the tumor is often undetected until the disease has spread and reached a terminal stage. Similarly, approximately 75% of women are diagnosed with
20 ovarian cancer after the disease has already reached an advanced stage (stage III or IV) because the symptoms of ovarian cancer are often vague or "silent." Despite aggressive surgical intervention and new chemotherapeutic regimens, the overall 5-year survival rate for these women with advanced stage ovarian cancer has remained constant over the past 30 years, at approximately 15%. Conversely, women
25 diagnosed with cancer confined to the ovary (stage I) have an overall 5-year survival rate approaching 90%.

Clearly, there is a need for the early and improved detection and treatment of neoplasms (e.g., stomach adenocarcinoma, colorectal adenocarcinoma, squamous cell lung carcinoma, lung adenocarcinoma, squamous cell carcinoma of the
30 esophagus, adenocarcinoma of the pancreas, urothel carcinoma of the urinary bladder, renal cell carcinoma of the kidney, adenocarcinoma of the prostate, ductal

carcinoma of the breast, lobular carcinoma of the breast, adenocarcinoma of the ovary, or adenocarcinoma of the uterus), as this would increase the chance of treating the neoplasm and, thereby, lead to an improved prognosis for long-term survival.

5

Summary of the Invention

We have discovered a class of polypeptides that react with an epitope specific for neoplastic cells. These polypeptides are not only excellent diagnostic tools, but also can induce apoptosis of the neoplastic cells to which they bind. This latter characteristic results in a treatment for neoplastic diseases that lacks the side-effects of many existing therapeutics.

The present invention features polypeptides, such as monoclonal antibodies that may be used in the diagnosis and treatment of a neoplasm, as well as the antigen recognized by such polypeptides. Accordingly, the first aspect of the invention features a purified polypeptide that specifically binds to a neoplastic cell, but does not bind to a non-neoplastic cell. This purified polypeptide specifically binds to a diffuse-type stomach adenocarcinoma, an intestinal-type stomach adenocarcinoma, an adenocarcinoma of the colon, an adenocarcinoma of the lung, a squamous cell carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast, an adenocarcinoma of the ovary, and an adenocarcinoma of the uterus, and not to non-neoplastic cells of the same tissue type, and the purified polypeptide is substantially identical to the full-length sequence of SEQ ID NO:2.

25

In desirable embodiments of the first aspect of the invention, the polypeptide specifically binds to at least one of the following: lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), or lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393). Desirably, the polypeptide can induce apoptosis of the neoplastic cell, but not of the non-neoplastic cell or the

30

polypeptide can decrease proliferation of the neoplastic cell, but not of the non-neoplastic cell.

The second aspect of the invention features a purified polypeptide containing the amino acid sequence of SEQ ID NO:2 and the third aspect of the invention
5 features a purified polypeptide containing amino acids 16-22, 37-52, and 85-103 of SEQ ID NO:2.

In desirable embodiments of the second and third aspects of the invention, the purified polypeptide specifically binds to a diffuse-type stomach adenocarcinoma, an intestinal-type stomach adenocarcinoma, an adenocarcinoma of the colon, an
10 adenocarcinoma of the lung, a squamous cell carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast, an adenocarcinoma of the ovary, and/or an adenocarcinoma of the uterus,
15 and not to non-neoplastic cells of the same tissue type.

In other desirable embodiments of the first three aspects of the invention, the polypeptide is an antibody or a functional fragment thereof, for example, a monoclonal antibody or a functional fragment thereof. Examples of functional fragments include V_L , V_H , F_V , F_C , Fab , Fab' , and $F(ab')_2$ fragments. Further, the
20 purified polypeptide of the first three aspects of the invention desirably is also produced by the LM-1 cell line having DSMZ Deposit Accession No. DSM ACC2623.

The fourth aspect of the invention features a functional fragment of an antibody that includes amino acids 16-22, 37-52, and/or 85-103 of SEQ ID NO:2.
25 Desirably, this functional fragment is a functional fragment of a monoclonal antibody or a V_H chain of an antibody.

In the fifth aspect, the invention features a purified polypeptide that is specifically bound by an antibody, or functional fragment thereof, containing the sequence of SEQ ID NO:2. Desirably, the polypeptide of the fifth aspect of the
30 invention is a polypeptide expressed by lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ

accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383) or lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393), but not by non-neoplastic cells of the same tissue type. In another desirable embodiment of the fifth aspect, the polypeptide
5 has an apparent molecular weight of approximately 70 kDa using sodium dodecyl sulfate polyacrylamide gel electrophoresis. In further desirable embodiments, the polypeptide of the fifth aspect, if present on a cell and bound by the antibody or functional fragment thereof, results in apoptosis of the cell or results in a reduction in proliferation of the cell.

10 In the sixth aspect, the invention features an isolated nucleic acid molecule containing the sequence of SEQ ID NO:1 and in the seventh aspect, the invention features an isolated nucleic acid molecule including nucleic acids 46-66, 109-156, and/or 253-309 of SEQ ID NO:1.

The eighth aspect of the invention features a vector including the nucleic acid
15 sequence of the sixth or seventh aspects of the invention. In the ninth aspect, the invention features an isolated cell containing the vector of the eighth aspect.

The tenth aspect of the invention features an isolated cell, e.g., a mammalian cell, which expresses the polypeptide of any one of the first three aspects of the invention. In a desirable embodiment of the tenth aspect, the mammalian cell is a
20 human cell. In additional desirable embodiments, the polypeptide is an antibody such as a monoclonal antibody or an IgM antibody.

In the eleventh aspect, the invention features a method of producing the purified polypeptide of any one of the first three aspects of the invention. This method involves contacting a cell with the vector of the eighth aspect and isolating the
25 polypeptide expressed by the cell.

The twelfth aspect of the invention features use of the polypeptide of any one of the first three aspects of the invention in a method of diagnosing a neoplasm in a mammal, e.g., a human. This method involves the steps of, (a) contacting a cell or tissue sample derived from the mammal with the purified polypeptide of any one of
30 the first three aspects of the invention, and (b) detecting whether the purified polypeptide binds to the cell or tissue sample, where binding of the purified

polypeptide to the cell or tissue sample is indicative of the mammal having a neoplasm. In a desirable embodiment of the twelfth aspect, the cell or tissue is derived from a stomach, a colon, a lung, an esophagus, a pancreas, a urinary bladder, a kidney, a prostate, a female breast, an ovary, or a uterus. Desirably, the polypeptide
5 used in the twelfth aspect of the invention is an antibody, such as a monoclonal antibody. In other desirable embodiments of the twelfth aspect, the polypeptide is conjugated to a detectable agent, for example, a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, or a growth inhibitor. In addition, this detectable agent desirably is capable of inducing apoptosis of the cell. In further desirable
10 embodiments of the twelfth aspect of the invention, the polypeptide is conjugated to a protein purification tag, such as a cleavable protein purification tag.

In the thirteenth aspect, the invention features a method of treating a proliferative disorder in a mammal, e.g., a human, using the purified polypeptide of any one of the first three aspects of the invention. This method involves the step of
15 contacting a cell with the purified polypeptide of any one of the first three aspects of the invention, where binding of the purified polypeptide to the cell results in a reduction in proliferation of the cell. This proliferative disorder may be, for example, a diffuse-type stomach adenocarcinoma, an intestinal-type stomach adenocarcinoma, an adenocarcinoma of the colon, an adenocarcinoma of the lung, a squamous cell
20 carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast, an adenocarcinoma of the ovary, or an adenocarcinoma of the uterus. In desirable
25 embodiments of the thirteenth aspect, the polypeptide is an antibody, for example, a monoclonal antibody.

In other desirable embodiments of the thirteenth aspect, the polypeptide is conjugated to a detectable agent selected from the group consisting of a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, and a growth inhibitor.
30 Desirably, this detectable agent is capable of inhibiting cell proliferation of the cell. In further desirable embodiments of this aspect of the invention, the polypeptide is

conjugated to a protein purification tag, such as a cleavable protein purification tag.

In the fourteenth aspect, the invention features a medicament including the purified polypeptide of any one of the first three aspects of the invention in a pharmaceutically acceptable carrier and, in the fifteenth aspect, the invention features
5 a diagnostic agent containing the purified polypeptide of any one of the first three aspects of the invention.

Definitions

By "detectable agent" is meant a compound that is linked to a diagnostic agent
10 to facilitate detection. Such a "detectable agent" may be covalently or non-covalently linked to a diagnostic agent. In addition, the linkage may be direct or indirect. Examples of "detectable agents" include, protein purification tags, cytotoxins, enzymes, paramagnetic labels, enzyme substrates, co-factors, enzymatic inhibitors, dyes, radionuclides, chemiluminescent labels, fluorescent markers, growth inhibitors,
15 cytokines, antibodies, and biotin.

By a "diagnostic agent" is meant a compound that may be used to detect a neoplastic cell by employing any one of the assays described herein as well as any other method that is standard in the art. A diagnostic agent may include, for example, an antibody which specifically binds to at least one of the following cells: lung
20 adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), and lung squamous carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393), but not to non-neoplastic cells. In addition, a "diagnostic agent" may inhibit cell
25 proliferation, induce apoptosis, or both only when it is bound to a neoplastic cell, but not a non-neoplastic cell.

Examples of neoplastic cells that may be detected with such a "diagnostic agent" include stomach adenocarcinoma, colorectal adenocarcinoma, squamous cell lung carcinoma, lung adenocarcinoma, squamous cell carcinoma of the esophagus,
30 adenocarcinoma of the pancreas, urothel carcinoma of the urinary bladder, renal cell carcinoma of the kidney, adenocarcinoma of the prostate, ductal carcinoma of the

breast, lobular carcinoma of the breast, adenocarcinoma of the ovary, and adenocarcinoma of the uterus cells. Moreover, a "diagnostic agent" may include, for example, peptides, polypeptides, synthetic organic molecules, naturally-occurring organic molecules, nucleic acid molecules, and components thereof, as well as one or more detectable agent covalently or non-covalently linked to the diagnostic agent.

By a "functional fragment," as used herein in reference to polypeptide, is meant a fragment that retains at least one biological activity of the full-length polypeptide. Examples of such a biological activity are the ability to specifically bind an antigen, induce apoptosis, and/or inhibit cell proliferation. These biological activities may be determined, for example, using any one of the assays described herein.

Examples of functional fragments of an antibody are V_L , V_H , F_V , F_C , Fab, Fab', or $F(ab')_2$ fragments (see, e.g., Huston et al., Cell Biophys. 22:189-224, 1993; and Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y., 1999). Desirably, a "functional fragment" has an amino acid sequence that is substantially identical to a fragment, e.g., 3, 4, 5, 10, 15, 20, 15, 30, 50, 75, or 100 contiguous amino acids, of the amino acid sequence of SEQ ID NO:2. In more desirable embodiments, a "functional fragment" is identical to a fragment of the sequence of SEQ ID NO:2. Such a "functional fragment" may contain 3, 4, 5, 10, 15, 20, 15, 30, 50, 75, or 100 contiguous amino acids of SEQ ID NO:2, or may be the entire amino acid sequence of SEQ ID NO:2. In desirable embodiments, such a fragment includes one or more of the Complement Determining Regions (CDR) of the V_H region of the LM-1 antibody. For example, a functional fragment may include 16-22, 37-52, and/or 85-103 of SEQ ID NO:2.

By "high stringency hybridization conditions" is meant, for example, hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 1% SDS, 2X SSC, 10% Dextran Sulfate, a first wash at approximately 65°C in about 2X SSC, 1% SDS, followed by a second wash at approximately 65°C in about 0.1X SSC. Alternatively, "high stringency hybridization conditions" may include hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 0.5% SDS, 5X SSPE, 1X

Denhardt's, followed by two washes at room temperature in 2X SSC, 0.1% SDS, and two washes at between 55-60°C in 0.2X SSC, 0.1% SDS.

A "hybridoma," as used herein, is any cell that is artificially created by the fusion of a normal cell such as an activated lymphocyte with a neoplastic cell, e.g., a myeloma. The hybrid cell, which results from the fusion of at least two cells, may produce a monoclonal antibody or T cell product identical to those produced by the immunologically-competent parent. In addition, these cells, like the neoplastic parent, are immortal.

"Inhibiting cell proliferation," as used herein, refers to a reduction in the rate of cell division of a cell in comparison with the normal rate of cell division of that type of cell. Inhibition of cell proliferation may be assayed using a number of methods standard in the art, for example, the MTT cell proliferation assay described herein, BrdU incorporation, and ³H thymidine uptake. Such assays are described, for example, in Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001. Desirably, the inhibition of cell proliferation is 20%, 40%, 50%, or 75%. In desirable embodiments, the inhibition of cell proliferation is 80%, 90%, 95%, or even a complete inhibition of cell proliferation.

"Inducing apoptosis," as used herein, refers to the appearance of characteristics in a cell that are well defined in the art (see, e.g., Wyllie *et al.*, *Br. J. Cancer* 80 Suppl. 1:34-37, 1999; Kerr *et al.*, *Br. J. Cancer* 26:239-257, 1972). These characteristics include morphological characteristics, such as membrane blebbing, DNA condensation, as well as changes in F-actin content, mitochondrial mass, and membrane potential. The induction of apoptosis may be assayed using a number of methods standard in the art, for example, a cell death ELISA, TUNEL staining, DNA stains, e.g., Hoechst 33258, and staining with various vital dyes such as acridine orange, Mito Tracker Red[®] staining (Molecular Probes, Eugene, OR), and Annexin V[®] staining (Becton Dickinson, NJ). As used herein "inducing apoptosis" refers to an increase in the number of cells undergoing apoptosis when compared with a control cell population. For instance, the increase of apoptosis may be 10%, 20%, 40%, 50%,

or 75%. In desirable embodiments, the induction of apoptosis results in an increase of apoptosis that is 2-fold, 3-fold, 10-fold, or even 100-fold over that seen in a control cell population.

5 A "neoplastic cell," as used herein, refers to a cell which is undergoing cell division, not undergoing apoptosis, or both, under inappropriate conditions. For example, a "neoplastic cell" may undergo cell division when a corresponding non-neoplastic cell does not undergo cell division, or, alternatively, a "neoplastic cell" may not respond to normal cell-cycle checkpoint controls.

10 A "proliferative disease," as used herein, refers to any disorder that results in the abnormal proliferation of a cell. Specific examples of proliferative diseases are various types of neoplasms, such as stomach adenocarcinoma, colorectal adenocarcinoma, squamous cell lung carcinoma, lung adenocarcinoma, squamous cell carcinoma of the esophagus, adenocarcinoma of the pancreas, urothel carcinoma of the urinary bladder, renal cell carcinoma of the kidney, adenocarcinoma of the
15 prostate, ductal carcinoma of the breast, lobular carcinoma of the breast, adenocarcinoma of the ovary, and adenocarcinoma of the uterus. However, proliferative diseases may also be the result of the cell becoming infected with a transforming virus.

A "protein purification tag," as used herein, is a peptide, e.g., an epitope tag,
20 that is covalently or non-covalently added to a protein to aid in the purification of the protein. Desirably such peptides bind with high affinity to an antibody or to another peptide such as biotin or avidin. Commercially available examples of epitope tags include His-tags, HA-tags, FLAG[®]-tags, and c-Myc-tags. However, any epitope that is recognized by an antibody also may be used as a protein purification tag. See, for
25 example, Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001. Protein purification tags may be cleaved from a protein, for example, by using an enzyme, e.g., thrombin, or a chemical, e.g., cyanogen bromide.

30 By "specifically recognizes" or "specifically binds," as used herein in reference to a polypeptide, e.g., an antibody, is meant an increased affinity of a

polypeptide for a particular protein, e.g., an antigen, relative to an equal amount of any other protein. For example, an antibody, e.g., the LM-1 human monoclonal antibody, that specifically binds to lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), and lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393), cells desirably has an affinity for its antigen that is least 2-fold, 5-fold, 10-fold, 30-fold, or 100-fold greater than for an equal amount of any other antigen, including related antigens. Binding of a polypeptide to another polypeptide may be determined as described herein, and by any number of standard methods in the art, e.g., Western analysis, ELISA, or co-immunoprecipitation.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, 75%, 80%, 85%, or 90% identity to a reference amino acid (e.g., the sequence of SEQ ID NO:2) or nucleic acid sequence (e.g., the sequence of SEQ ID NO:1), or a fragment thereof. In desirable embodiments, the polypeptide or nucleic acid sequence is at least 95%, 98%, 99%, or even 100% identical to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 3, 4, 5, 6, 8, 10, or 15 amino acids and desirably at least 20 or 25 contiguous amino acids. In more desirable embodiments, the length of comparison sequences is at least 30, 50, 75, 90, 95, or 100 contiguous amino acids, or even the full-length amino acid sequence. For nucleic acids, the length of comparison sequences will generally be at least 9, 10, 15, 20, or 25 contiguous nucleotides, and desirably at least 30 contiguous nucleotides. In more desirable embodiments, the length of comparison sequences is at least 50, 75, 150, 225, 270, 285, or 300 contiguous nucleotides, or even the full-length nucleotide sequence.

Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups:

glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Multiple sequences may also be aligned using the Clustal W(1.4) program (produced by Julie D. Thompson and Toby Gibson of the European Molecular
5 Biology Laboratory, Germany and Desmond Higgins of European Bioinformatics Institute, Cambridge, UK) by setting the pairwise alignment mode to "slow," the pairwise alignment parameters to include an open gap penalty of 10.0 and an extend gap penalty of 0.1, as well as setting the similarity matrix to "blosum." In addition, the multiple alignment parameters may include an open gap penalty of 10.0, an extend
10 gap penalty of 0.1, as well as setting the similarity matrix to "blosum," the delay divergent to 40%, and the gap distance to 8.

By "purified" or "isolated" is meant separated from other components that naturally accompany it. Typically, a factor is substantially pure when it is at least 50%, by weight, free from proteins, antibodies, and naturally-occurring organic
15 molecules with which it is naturally associated, or in reference to a nucleic acid molecule, is free from the nucleic acid sequences that naturally flank the sequence of the nucleic acid molecule in the genome of an organism. Desirably, the factor is at least 75%, more desirably, at least 90% or 95%, and most desirably, at least 99%, by weight, pure. A substantially pure factor may be obtained by chemical synthesis,
20 separation of the factor from natural sources, or production of the factor in a recombinant host cell that does not naturally produce the factor. Proteins, vesicles, and organelles may be purified by one skilled in the art using standard techniques, such as those described by Ausubel *et al.* (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). The factor is desirably at least 2, 5, or 10 times
25 as pure as the starting material, as measured using polyacrylamide gel electrophoresis, column chromatography, optical density, HPLC analysis, or Western analysis (Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). Desirable methods of purification include immunoprecipitation, column chromatography such as immunoaffinity chromatography and nickel affinity columns,
30 magnetic bead immunoaffinity purification, and panning with a plate-bound antibody.

By "vector" or "expression vector" is meant an expression system, a viral vector, a nucleic acid-based shuttle vehicle, a nucleic acid molecule adapted for nucleic acid delivery, or an autonomous self-replicating circular DNA (e.g., a plasmid). When a vector is maintained in a host cell, the vector can either be stably
5 replicated by the cell during mitosis as an autonomous structure, incorporated into the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

Other features and advantages of the invention will be apparent from the following Detailed Description, the Drawings, and the Claims.

10

Brief Description of the Drawings

Figures 1A-1D are a series of images showing immunohistochemical staining of monoclonal antibody LM-1 on different carcinomas. Paraffin sections were stained with hematoxylin-eosin, positive control antibodies (CK5/6 for squamous cell carcinoma of the lung and esophagus, AE1/AE3 for adenocarcinoma of
15 the colon, and CK8 for invasive ductal adenocarcinoma of the breast), secondary antibody alone as a negative control, and antibody LM-1. Figure 1A shows staining of a squamous cell carcinoma of the lung; Figure 1B shows staining of an adenocarcinoma of the colon; Figure 1C shows staining of an esophageal squamous cell carcinoma; and Figure 1D shows staining of an invasive ductal carcinoma of the
20 breast. The original magnification for these images was 200x.

Figure 2 is a graph depicting the functional analysis of antibody LM-1 *in vitro*. The consequences of antibody treatment on the proliferation of different carcinoma cell lines were measured using a 3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide ("MTT") proliferation assay. Figure 2 shows the
25 concentration dependent inhibition of cell proliferation with antibodies LM-1 on lung pancreas carcinoma cell line LOU-NH91. The control for these experiments was depleted cell culture supernatant with an unrelated IgM antibodies added at similar concentrations.

Figures 3A and 3B are a series of graphs of the results of MTT reduction
30 assays for mitochondrial dehydrogenase activity showing that the LM-1 monoclonal antibody inhibits cell proliferation and decreases survival, or induces apoptosis of

EPLC-272H epidermoid cell carcinoma of the lung cells after 24 hours of incubation (Figure 3A) and after 48 hours of incubation (Figure 3B).

Figure 4 is a graph showing that the LM-1 antibody induces apoptosis. In these experiments, apoptosis was detected using the Cell Death Detection ELISA^{PLUS} apoptosis assay. Figure 4 shows LM-1 monoclonal antibody-induced apoptosis of lung carcinoma cell line LOU-NH91. The control in these experiments was depleted cell culture supernatant at a similar concentration.

Figures 5A and 5B are a series of graphs of the results of a cell death ELISA showing that the LM-1 monoclonal antibody induces apoptosis of LOU-NH91 cells after 24 hours of incubation (Figure 5A) and after 48 hours of incubation (Figure 5B).

Figure 6 is a Western blot showing the proteins recognized by human monoclonal antibody LM-1 on membrane extracts of lung carcinoma cell line LOU-NH91. Antibody LM-1 recognizes one main band with a molecular weight of approximately 70 kDa. As a control in these experiments, unrelated human IgM was added at a similar concentration to rule out non-specific binding.

Figure 7 is the nucleic acid sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of the variable region of the heavy chain of human monoclonal antibody LM-1. Complement Determining Regions (CDR) 1-3 and the D and J regions are also shown.

Detailed Description

The present invention features polypeptides, such as antibodies, and their use in the treatment and diagnosis of neoplasms. We have characterized human monoclonal antibody (LM-1) that specifically recognizes a number of carcinomas. Not only does this monoclonal antibody recognize these neoplasms, but, upon binding to a cell, it can induce apoptosis of neoplastic cells, inhibit their proliferation, or even both. Thus, the LM-1 monoclonal antibody, and other antibodies, or fragments thereof, that are specific for the antigen recognized by this antibody, may be used in a variety of methods for diagnosing and treating a neoplasm.

The cell line that produces the human LM-1 monoclonal antibody was deposited on November 6, 2003 at the German Collection of Microorganisms and Cell

Cultures ("DSMZ" - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany) under the terms of the Budapest Treaty and has been assigned Accession Number DSM ACC2623.

5 Antibodies and Polypeptides

Antibodies play an essential role in maintaining the health of an individual. In particular, antibodies are present in serum and bind to and help eliminate diverse pathogens such as bacteria, viruses, and toxins. Antibodies consist of Y-shaped protein structures built from two heavy chains and two light chains. Each chain has a modular construction: each light chain consists of two domains, and each heavy chain has at least four domains. The antigen binding site is fashioned by one domain from the heavy chain (V_H domain) and one domain from the light chain (V_L domain). Indeed, small antigen binding fragments can be prepared by linking these two domains, either associated non-covalently, or covalently via disulphide bonds or a peptide linker. The antigen binding domains are more variable in amino acid sequence than the other domains of the antibody, and are therefore termed variable (V) domains, in contrast to the constant (C) domains. The constant domains of the antibody are responsible for triggering antibody effector mechanisms, such as complement lysis and cell-mediated killing.

20 Antibodies are made by B-lymphocytes in a process involving gene rearrangement. During the development of these cells, the genes encoding the variable domains are assembled from genetic elements. In the case of the V_H domains there are three elements, the un-rearranged V_H gene, D segment, and J_H segment. In the case of the V_L domains, there are two elements, the un-rearranged V_L (V Lambda or V Kappa) gene and the J_L (J Lambda or J Kappa) segment. Random combination of these gene segments and random combination of the rearranged V_H and V_L domains generate a large repertoire of antibodies, capable of binding to a large diversity of equally diverse antigens. Further, the V_H and V_L regions each have three Complement Determining Regions (CDR) and four framework regions (FR). The FRs are the backbone of the antibody and the CDRs are the parts of the antibody that bind the

antigen. One skilled in the art can determine the FR and CDR regions of an antibody by comparing the amino acid sequence of a number of antibodies raised in the same species.

In general, the presently claimed polypeptide is any agent that binds to any one
5 of lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung
adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid
lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), and lung
squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393),
but does not bind to non-neoplastic cells. The polypeptide may be an antibody, such
10 as a human monoclonal antibody (e.g., LM-1 or a functional fragment thereof).
Overall, the polypeptide of the invention can exclusively bind to both neoplastic
tissues and neoplastic cells, but not to non-neoplastic tissue or cells. The polypeptide
also may induce apoptosis of a neoplastic cell to which it binds, but not in a non-
neoplastic cell, or, alternatively, the polypeptide may inhibit proliferation of the
15 neoplastic cell it binds to, but not in a non-neoplastic cell. Desirably, the polypeptide
can simultaneously induce apoptosis and inhibit proliferation of neoplastic cells, but
not of non-neoplastic cells. Such a polypeptide is, therefore, useful for the detection,
monitoring, prevention, and treatment of cancers in mammals. Exemplary cancers
amenable to the methods of the current invention include colorectal cancer, ovarian
20 carcinoma, squamous cell lung carcinoma, small cell lung carcinoma, lobular and
ductal mammary carcinomas, melanoma, breast cancer, lung cancer, such as lung
adenocarcinomas, gastric cancer, pancreatic cancer, such as pancreatic
adenocarcinomas, glioma, sarcomas, gastrointestinal cancer, brain tumor, esophageal
cancer, such as esophageal squamous cell carcinomas, stomach cancer, osteosarcoma ,
25 fibrosarcomas, urinary bladder cancer, prostate cancer, such as prostate
adenocarcinomas, renal cancer, ovarian cancer, testicular cancer, endometrial cancer,
cervical cancer, uterine adenocarcinomas, Hodgkin's disease, lymphomas, and
leukemias. Such polypeptides are particularly useful for the detection and treatment
of a stomach adenocarcinoma, colorectal adenocarcinoma, squamous cell lung
30 carcinoma, lung adenocarcinoma, squamous cell carcinoma of the esophagus,
adenocarcinoma of the pancreas, urothel carcinoma of the urinary bladder, renal cell

Once constructed, hybridomas are generally stable in growth and antibody production in standard and mass cultures (flasks, miniPerm, fermenters, etc.) for several months. Levels of antibody production typically range between 0.01-0.1 mg/mL in flasks and between 0.1-0.5 mg/mL in miniPerm. Cell fusion may be achieved by any method
5 known in the art, and includes, for example, the use of 40% polyethylene glycol. Hybridomas may be cultured in media containing HAT (Hypovanthin-aminopterin-thymidine) and after four weeks, supernatants may be screened for antibody production using an ELISA assay. Positive clones may then be tested in attachment inhibition and binding assays using autologous cell lines as prepared above. Positive
10 clones further may be tested using immunoperoxidase staining of tumor and normal tissues. Thus, clones may be selected on the basis of their reactivity with autologous and allogeneic neoplastic cells. The antibody may be purified from mass cultures with use of cation-exchange chromatography followed by gel filtration as described, for example, by Vollmers et al. (Oncology Reports 5:35-40, 1998). Following the
15 production of antibodies, additional functional and immunohistochemical tests of the antibodies produced by the trioma may be performed. For example, the antibodies produced by the hybridoma can be tested for their ability to induce apoptosis, inhibit cellular proliferation, or both, relative to untreated control cells. The antibodies can also be tested for their ability to specifically bind the neoplastic cell lines e.g., lung
20 adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), and lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393), relative to non-neoplastic cells.

25 Alternatively, the polypeptide, including an antibody, or a fragment thereof, may be produced by the expression of the polypeptide or antibody in a host cell such as *E. coli* or yeast, e.g., *S. cerevisiae*, or a mammalian cell line. For example, an antibody of the invention may be identified as follows. A nucleic acid sequence encoding an antibody, or a fragment thereof, may be inserted into filamentous
30 bacteriophage to generate libraries of approximately 10^7 or more antibodies. Each phage expresses an antibody on its surface that is encoded by the nucleic acid it

carcinoma of the kidney, adenocarcinoma of the prostate, ductal carcinoma of the breast, lobular carcinoma of the breast, adenocarcinoma of the ovary, or adenocarcinoma of the uterus.

5 Production

The polypeptides according to the claimed invention can be produced by any method known in the art for small scale, large scale, or commercial production of polypeptides. For example, monoclonal antibodies, such as LM-1, may be produced by hybridoma cell lines. Such cell lines are typically generated by the fusion of spleen
10 and lymph node lymphocytes derived from patients having a neoplasm, such as colon carcinoma or a pancreatic carcinoma, with a heteromyeloma cell line. Exemplary heteromyeloma cell lines include, for example, HAB-1 (Vollmers et al, *Cancer* 74:1525-1532, 1994), CB-F7 (Delvig et al., *Hum. Antibodies Hybridomas* 6:42-46, 1995), K6H6B5 (Delvig et al., *Hum. Antibodies Hybridomas* 6:42-46, 1995),
15 H7NS.934 (Delvig et al., *Hum. Antibodies Hybridomas* 6:42-46, 1995), SHM-D33 (Bron et al., *Proc. Natl. Acad. Sci. USA* 81:3214-3217, 1984), and B6B11 (Borisova et al., *Vopr. Virusol.* 44:172-174, 1999). The ability to generate human monoclonal antibodies from lymphocytes of cancer patients allows the isolation of antibodies that are generated by an immune response in the cancer patient to the tumor.

20 Typically, portions of the lymph nodes or spleen are surgically removed from a patient having cancer, such as colon carcinoma or a pancreatic carcinoma. Lymphocytes may be prepared as cell suspensions by mechanical means and subsequently fused at, for example, a 1:2 or 1:3 ratio with a heteromyeloma cell line under conditions that result in cell fusion. For instance, the heteromyeloma cell line
25 HAB-1, which is generated by the fusion of a human lymphocyte with the mouse myeloma NS-0, may be used for this purpose. A proportion of lymphocytes isolated from the cancer patient may also be maintained in culture. These cells serve as a source of human autologous cells useful for the initial antibody screening described below.

30 Following the fusion of the lymphocytes derived from the cancer patient with the heteromyeloma cell line, an antibody producing hybridoma or trioma is generated.

contains. Antibodies of the invention may thus be screened and detected by functional and histochemical assays as described herein, and such genes may be subsequently selected and expressed in *E.coli*. This system is described, for example, in U.S. Patent No. 5,876,691.

5 Antibodies, or functional fragments thereof, may also be generated using, for example, direct synthesis using recombinant methods. These methods are standard in the art. For example, a nucleic acid sequence may be amplified using the polymerase chain reaction (PCR). The PCR technique is known in the art and is described, for example in U.S. Patent No. 4,683,195. Using standard methods, and as described
10 herein, the sequence of a monoclonal antibody expressed by a hybridoma may be obtained and functional fragments of the antibody may be amplified. For example, whole RNA may be isolated from a hybridoma expressing a tumor-specific monoclonal antibody. cDNA may then be generated from the RNA using reverse transcriptase and the cDNAs which contain the functional fragments of the variable
15 regions of the heavy and light chains may be amplified using PCR. The PCR products may then be purified and cloned into expression vectors, e.g., plasmid or viral vectors. Many standard vectors are available and the selection of the appropriate vector will depend on, for example, the size of the DNA inserted into the vector and the host cell to be transfected with the vector.

20 The nucleic acid molecules of the invention may be expressed in a variety of standard vectors and host cells. Any promoter that is active in the host cell may be used to express a nucleic acid molecule. Nonetheless, for expression of an antibody or a fragment of an antibody in a mammalian cell, use of an immunoglobulin gene promoter is desirable. Methods of introducing a vector into a host cell are standard in
25 the art and include, electroporation, use of synthetic lipid polymers, e.g., Lipofectin™, use of calcium chloride, and use of DEAE Dextran. Such methods are also described in, for example, Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001.

30

Isolation of Amino Acid Variants of a Polypeptide

Amino acid sequence variants of a polypeptide, such as an antibody, e.g., an LM-1 antibody, can be prepared by introducing appropriate nucleotide changes into the DNA encoding the antibody, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence of the LM-1 antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., the ability to induce apoptosis of a neoplastic cell, but not a non-neoplastic cell, or the ability to inhibit the proliferation of a neoplastic cell, but not a non-neoplastic cell. The amino acid changes also may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants of a polypeptide, such as an antibody, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, or deleting the target residue.

A useful method for identification of specific residues or regions for mutagenesis in a polypeptide is called "alanine scanning mutagenesis" and is described, for example, by Cunningham and Wells (Science 244:1081-1085, 1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most desirably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. The domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation need not be predetermined. For instance, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted

at the target codon or region and the expressed variants are screened for, e.g., the ability to induce apoptosis of a neoplastic cell and not a non-neoplastic cell, or to inhibit the proliferation of a neoplastic cell and not a non-neoplastic cell.

The sites of greatest interest for substitutional mutagenesis include sites identified as affecting the biological activity of a polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, may be substituted in a relatively conservative manner. For instance, ala may be substituted with val, leu, or ile; arg may be substituted with lys, gln, or asn; asn may be substituted with gln, his, lys, or arg; asp may be substituted with glu; cys may be substituted with ser; gln may be substituted with asn; glu may be substituted with asp; gly may be substituted with pro; his may be substituted with asn, gln, lys, or arg; ile may be substituted with leu, val, met, ala, or phe; leu may be substituted with ile, val, met, ala, or phe; lys may be substituted with arg, gln, or asn; met may be substituted with leu, phe, or ile; phe may be substituted with leu, val, ile, or ala; pro may be substituted with gly; ser may be substituted with thr; thr may be substituted with ser; trp may be substituted with tyr; tyr may be substituted with trp, phe, thr, or ser; and val may be substituted with ile, leu, met, or phe.

Conjugation of the Antibody with a Detectable Agent

If desired, the claimed polypeptide such as an antibody (e.g., monoclonal antibody LM-1), or a functional fragment thereof, may be linked to a detectable agent to facilitate the purification of the polypeptide as well as the diagnosis, monitoring, or treatment of cancer in a mammal in need thereof. The selection of suitable detectable agent will depend on the intended use of the polypeptide and will be apparent to those of ordinary skill in the art. Detectable agents according to the claimed invention include, for example, protein purification tags, cytotoxins, enzymes, paramagnetic labels, enzyme substrates, co-factors, enzyme inhibitors, dyes, radionuclides, chemiluminescent labels, fluorescent markers, growth inhibitors, and biotin.

A protein purification tag may be conjugated to the polypeptide of the invention, to facilitate isolation of the polypeptide. Examples of tags that can be used include His-tags, HA-tags, FLAG[®]-tags, and c-Myc tags. An enzymatic or chemical

cleavage site may be engineered between the polypeptide and the tag moiety so that the tag can be removed following purification. Suitable toxins include diphtheria toxin, Pseudomonas exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholinesterase. Examples of suitable radioisotopic labels include ^3H , ^{125}I , ^{131}I , ^{32}P , ^{35}S , and ^{14}C . Desirably, the radioisotope will emit in the 10-5,000 kev range, more desirably 100-500 kev. Paramagnetic isotopes may also be conjugated to the polypeptide and used *in vivo* for the diagnosis and treatment of cancer. The use of such conjugated antibodies may be for *in vivo* nuclear magnetic resonance imaging. Such a method has previously been described (see, for example, Schaefer et al., JACC 14:472-480, 1989; Shreve et al., Magn. Reson. Med. 3:336-340, 1986; Wolf, Physiol. Chem. Phys. Med. NMR 16:93-95, 1984; Wesbey et al., Physiol. Chem. Phys. Med. NMR 16:145-155, 1984; and Runge et al., Invest. Radiol. 19:408-415, 1984). Alternatively, the radiolabeled antibody may also be used in radioimmunoguided surgery (RIGS), which involves the surgical removal of any tissue the labeled antibody binds to. Thus, the labeled antibody guides the surgeon towards neoplastic tissue by distinguishing it from non-neoplastic tissue. Radiolabels useful for tumor imaging are preferably short-lived radioisotopes. Various radioactive metals with half-lives ranging from 1 hour to 11.4 days are available for conjugation to antibodies, such as scandium-47 (3.4 days), gallium-67 (2.8 days), gallium-68 (68 minutes), technetium-99m (6 hours), indium-111 (3.2 days), and radium-223 (11.4 days), of which gallium-67, technetium-99m, and indium-111 are preferable for gamma camera imaging, gallium-68 is preferable for positron emission tomography, and scandium-47 and radium-223 (and other alpha-emitting radionuclides) are preferable for tumor therapy.

Examples of suitable fluorescent markers include fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde, and fluorescamine. Examples of chemiluminescent markers include a luminal label,

isoluminal label, aromatic acridinium ester label, imidazole label, acridinium salt label, oxalate ester label, luciferin label, luciferase label, and aequorin label. Those of ordinary skill in the art would know of other suitable labels, which may be employed in accordance with the present invention. Conjugation of these detectable agents to the
5 claimed polypeptides such as monoclonal antibodies, or fragments thereof, can be accomplished using standard techniques commonly known in the art. Typical antibody conjugation techniques are described by Kennedy et al. (*Clin. Chim. Acta* 70, 1-31, 1976) and Schurs et al. (*Clin. Chim. Acta* 81, 1-40, 1977) and include, for example, the glutaraldehyde method, the periodate method, the dimaleimide method,
10 the m-maleimidobenzyl-N-hydroxy-succinimide ester method. Antibodies may be radiolabeled by any of several techniques known to the art, described, for example, in U.S. patent No. 4,444,744. All of these methods are incorporated by reference herein.

In all aspects of the present invention, it is understood that mixtures of
15 different or the same labeled polypeptides specific to different antigens or different epitopes of the same antigen associated with the same or different tumor or tumor cell types may be used. Such a combination may enhance detection, localization and/or therapy in certain cases, and can also increase the range of a broad screen for more than one neoplasm or type of neoplasm.

20

Polypeptides Conjugated to Anti-Tumor Agents

Although the polypeptide of the invention may induce apoptosis of neoplastic cells, inhibit cellular proliferation of neoplastic cells, or both, the polypeptide may in addition be conjugated to an agent that kills neoplastic cells or that inhibits their
25 proliferation. The targeting ability of the polypeptide, such as an antibody or fragment thereof, results in the delivery to deliver of the cytotoxic or anti-proliferative agent to the tumor to enhance the destruction of the tumor. The polypeptide therefore may be used for the treatment and prevention of cancer in a mammal, such as a human patient. The cytotoxic agent linked to the polypeptide may be any agent that destroys or

30

damages a tumor cell or tumor to which the polypeptide has bound. Examples of such agents include chemotherapeutic agents or radioisotopes, enzymes which activates a pro-drug, or a cytokine.

Suitable chemotherapeutic agents are known to those skilled in the art and include, for example, taxol, mithramycin, deoxyco-formycin, mitomycin-C, L-asparaginase, interferons (especially IFN-alpha), etoposide, teniposide, anthracyclines (e.g., daunomycin and doxorubicin), methotrexate, vindesine, neocarzinostatin, cis-platinum, chlorambucil, cytosine arabinoside, 5-fluorouridine, melphalan, ricin, and calicheamicin. The chemotherapeutic agents may be conjugated to the antibody using conventional methods known in the art.

Suitable radioisotopes for use as cytotoxic agents are also known to those skilled in the art and include, for example, ^{131}I , or an astatine such as ^{211}At . These isotopes may be attached to the polypeptide, either covalently or non-covalently, using conventional techniques known in the art.

Alternatively, the cytotoxic agent may also be an enzyme, which activates a pro-drug. This allows the conversion of an inactive pro-drug to its active, cytotoxic form at the tumor site and is called "antibody-directed enzyme pro-drug therapy" (ADEPT). Thus, the polypeptide-enzyme conjugate may be administered to the patient and allowed to localize in the region of the tumor to be treated. The pro-drug is then administered to the patient such that conversion to the cytotoxic drug is localized in the region of the tumor to be treated under the influence of the localized enzyme. An exemplary enzyme is bacterial carboxypeptidase G2 (CPG2) the use of which is described in, for example, WO 88/07378. The polypeptide-enzyme conjugate may, if desired, be modified in accordance with the teaching of WO 89/00427, such as to accelerate its clearance from areas of the body that are not in the vicinity of a neoplasm. The polypeptide-enzyme conjugate may also be used in accordance with WO 89/00427, for example, by providing an additional component, which inactivates the enzyme in areas of the body that are not in the vicinity of the tumor.

As another alternative, the cytotoxic agent conjugated to the claimed polypeptide may also be a cytokine such as interleukin-2 (IL-2), interleukin-4 (IL-4),

or tumor necrosis factor alpha (TNF-alpha). The polypeptide targets the cytokine to the tumor so that the cytokine mediates damage to or destruction of the tumor without affecting other tissues. The cytokine may be fused to the polypeptide at the DNA level using conventional recombinant DNA techniques.

- 5 In addition, any inhibitor of cell proliferation. e.g., genistein, tamoxifen, or cyclophosphamide, may be conjugated with a polypeptide of the invention.

Dosage

- With respect to the therapeutic methods of the invention, it is not intended that
- 10 the administration of the claimed polypeptide to a patient be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to decrease the number of neoplastic cells
- 15 by inducing apoptosis of neoplastic cells, by inhibiting proliferation of tumor cells, or both. The compound(s) may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one day, two days, one week, two weeks, or one month. For example, the polypeptide (e.g., monoclonal antibody LM-1, or a
- 20 functional fragment thereof) may be administered once a week for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The precise dose will vary dependent on the
- 25 polypeptide used, the density, on the tumor surface, of the ligand to which the polypeptide binds, and the rate of clearance of the polypeptide. For example, the dosage of the LM-1 antibody can be increased if the lower dose does not provide sufficient anti-neoplastic activity. Conversely, the dosage of the LM-1 antibody can be decreased if the neoplasm is cleared from the patient.

- 30 While the attending physician ultimately will decide the appropriate amount and dosage regimen, a therapeutically effective amount of the claimed polypeptide,

such as a monoclonal antibody or a fragment thereof, may be, for example, in the range of about 0.1 mg to 50 mg/kg body weight/day or 0.70 mg to 350 mg/kg body weight/week. Desirably a therapeutically effective amount is in the range of about 0.50 mg to 20.0 mg/kg, and more desirably in the range of about 0.50 mg to 15.0 mg/kg for example, about 0.2, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 8.5, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, or 15.0 mg/kg body weight administered daily, every other day, or twice a week.

For example, a suitable dose is an amount of the polypeptide that, when administered as described above, is capable of inducing apoptosis, and is at least 20% above the basal (i.e., untreated) level. In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. According to this invention, the administration of the polypeptide can induce neoplastic cell apoptosis by at least 20%, 40%, 50%, or 75% above that of an untreated control as measured by any standard assay known in the art. More desirably, apoptosis is induced by 80%, 90%, 95%, or even 100% above that of an untreated control. Alternatively, the administration of the polypeptide can inhibit neoplastic cell proliferation by at least 20%, 40%, 50%, or 75% below that of an untreated control as measured by any standard assay known in the art. More desirably, proliferation is inhibited by 80%, 90%, 95%, or even 100% below that of an untreated control. Most desirably, the polypeptide can simultaneously inhibit proliferation and induce apoptosis of neoplastic cells relative to untreated control cells. Such responses can be monitored by any standard technique known in the art. In general, for pharmaceutical compositions, the amount of antibody present in a dose ranges from about 25 µg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

Formulation of Pharmaceutical Compositions

The claimed polypeptide may be administered by any suitable means that results in a concentration having anti-neoplastic properties upon reaching the target region. The polypeptide may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneous, intravenous, intramuscular, or intraperitoneal) administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A.R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. If the neoplastic cells are in direct contact with the blood (e.g., leukemias), or if the tumor is only accessible by the bloodstream then the intravenous (I.V.) route may be used. In cases in which tumors grow in confined spaces such as the pleural cavity or the peritoneal cavity, the polypeptide may be directly administered into the cavity rather than into the blood stream. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation. Formulations can be found in Remington: The Science and Practice of Pharmacy, *supra*.

Diagnosis and Monitoring Cancer Progression

As discussed above, the present invention is directed to a method for detecting or diagnosing a neoplasm in a mammal, preferably a human patient. Typically, any neoplasm in which administration of the claimed polypeptide causes an induction in apoptosis or a reduction in proliferation are amenable to the methods of this invention.

The claimed polypeptides are particularly useful since they are specific to neoplasms or neoplastic cells, but not normal cells or tissue. Accordingly, this polypeptide can bind to neoplastic cells within the tumor, but not the normal surrounding tissue, thus allowing the detection, the treatment, or both, of a neoplasm in a mammal. For instance, one may use a polypeptide of the invention to determine 5 is a biopsy removed the entire tumor by verifying that no cells bound by the polypeptide remain in the patient or, by verifying that tumor removed from the patient is entirely surrounded by cells that are not bound by the polypeptide.

It is understood that to improve the sensitivity of detection, multiple neoplastic 10 markers may be assayed within a given sample or individual. Thus, polypeptides such as antibodies or functional fragments specific for different antigens may be combined within a single assay, or in multiple assays. Further, multiple primers or probes specific to neoplasms may be used concurrently. The selection of markers may be based on routine experiments to determine combinations that results in optimal 15 sensitivity.

In Vitro Detection of a Neoplasm

In general, the diagnosis of a neoplasm in a mammal involves obtaining a biological sample from the mammal (e.g., human patient), contacting such sample 20 with the polypeptide of the invention (e.g., a monoclonal antibody, such as LM-1, or a functional fragment thereof), detecting in the sample the level of reactivity or binding of the polypeptide to neoplastic cells relative to a control sample, which corresponds to non-neoplastic cells derived from healthy tissue from the mammal in which the cancer is being diagnosed or from another patient known not to have neoplasm. Thus, 25 the methods of this invention are particularly useful for the detection of early stage tumors or metastases, which are otherwise undetectable. Accordingly, in addition to diagnosing a neoplasm in a patient, the methods of this invention may also be used to monitor progression of a neoplasm in a mammal. The polypeptides described herein therefore may be used as markers for the progression of a neoplasm. For this purpose, 30 the assays described below, which are used for the diagnosis of a neoplasm, may be performed over time, and the change in the level of reactive polypeptide(s) evaluated.

For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a neoplasm is progressing in those patients in whom the level of bound polypeptide detected increases over time. In contrast, the neoplasm is not progressing when the level of bound polypeptide either remains constant or decreases with time. Alternatively, as is noted above, the polypeptide of the invention may also be used to determine the presence of tumor cells in the mammal following tumor resection by surgical intervention to determine whether the tumor has been completely removed from the mammal.

Desirably, the polypeptide is linked to a detectable agent, which facilitates detection, or measurement of polypeptide reactivity. The biological sample is any biological material, which may contain neoplastic cells and include, for example, blood, saliva, tissue, serum, mucus, sputum, urine, or tears. The biological sample may also be a tissue section, which may be fixed tissue, fresh tissue, or frozen tissues. A neoplasm is detected or diagnosed in the mammal from which the sample was obtained if there is an increase in the level of reactivity of the antibody with the biological sample over the control sample. Such increase is at least 10%, 20%, 30%, 40%, 50%, or more than 50% over control levels. The level of binding or reactivity can be determined by any method known in the art and is described in further detail below.

In Vitro Diagnostic Assays

The diagnosis of neoplasms using the claimed polypeptide may be performed by any method known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y., 1999. For example, the polypeptide may be used for enzyme-linked immunosorbent assay (ELISA), Western blotting or *in situ* detection of tumor cells in a tissue sample. For example, the ELISA assay typically involves the use of the polypeptide, such as an antibody, immobilized on a solid support to bind to the tumor cells in the biological sample. The bound tumor cell may then be detected using a detection reagent that

contains a reporter group and that specifically binds to the antibody/tumor cell complex. Such detection reagents include, for example, any binding agent that specifically binds to the antibody, such as an anti-immunoglobulin, protein G, protein A, or a lectin. Alternatively, a competitive assay may be utilized, in which the

5 polypeptide is an antibody and in which the antigens, to which the antibody is specific to is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the biological sample. The extent to which components of the sample inhibit the binding of the labeled antigens to the antibody is indicative of the reactivity of the sample with the immobilized antibody. Diagnosis of

10 a neoplasm in a patient may also be determined by a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized

15 polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group. For example, to determine the presence or absence of a neoplasm, such as colorectal

20 adenocarcinoma, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. The cut-off value for the detection of a neoplasm is the average mean signal obtained when the antibody is incubated with samples from patients without a neoplasm.

25 The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods may be used. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent

30 group or an enzyme).

Enzyme reporter groups may generally be detected by the addition of substrate (generally for a defined period of time), followed by spectroscopic or other analysis of the reaction products.

5 The polypeptides of the invention may also be employed histologically for *in situ* detection or quantitative determination of tumor cells, for example, by immunofluorescence or immunoelectron microscopy. *In situ* detection or determination may be accomplished by removing a tissue specimen from a patient and allowing a labeled antibody to bind to any tumor cell in the specimen. Using such a procedure not only allows the detection of neoplastic cells in a sample, but also allows
10 for the determination of their spatial distribution. As another example, the biological sample can be a smear of biological material containing neoplastic cells on a slide, and the detection of neoplastic cells in the biological material is achieved by examining the smear with a microscope or by fluocytometry.

15 *In Vivo detection of a Neoplasm*

Alternatively, the antibody of the invention may also be used *in vivo* for detecting and localizing a neoplasm. Such a method may involve injecting a mammal, desirably a human subject, parenterally with a polypeptide of the invention, such as monoclonal antibody LM-1, or a functional fragment thereof, which has been labeled
20 with a detectable agent, and is described, for instance, in U.S. Patent No. 4,444,744. For example, the polypeptide can be radiolabeled with a pharmacologically inert radioisotope and administered to the patient. The activity of the radioisotope can be detected in the mammal using a photoscanning device, and an increase in activity relative to a control reflects the detection and localization of a neoplasm.

25

Treatment

In addition to the diagnosis and monitoring of neoplasms in mammals, the present invention also features methods for treating neoplasms in a mammal, desirably a human patient. The method generally involves the administration of a biologically
30 effective amount of the polypeptide of the invention to the patient. The polypeptide is typically administered to the mammal by means of injection using any routes of

administration such as by intrathecal, subcutaneous, submucosal, or intracavitary injection as well as for intravenous or intraarterial injection. Thus, the polypeptide may be injected systemically, for example, by the intravenous injection of the polypeptide such as monoclonal antibody LM-1, or a functional fragment thereof, into the patient's bloodstream or alternatively, the polypeptide can be directly injected at the site of the neoplasm or at a location in proximity to the neoplastic cells.

In general, and as discussed above, binding of the polypeptide of the invention to neoplastic cells results in an induction in apoptosis, a reduction in cellular proliferation, or both relative to the control sample. Alternatively, the antibodies may also activate the complement pathway, which ultimately causes holes to be punctured into the cellular membrane, resulting in cell death.

If desired, the polypeptides may also be conjugated to drugs or toxins as described above. Once attached to the cell surface, the conjugate may be engulfed into the cell cytoplasm where cell enzymes cleave, and, thus, activate or free the drugs or toxins from the conjugate. Once released, the drugs or toxins damage the cell and irreversibly induce cell death. With respect to radiolabeled antibodies, binding to neoplastic cells and the resulting emission of radiation, at a short distance from the cell DNA, produces damage to the latter thus inducing cell death in the next replication round. For example, after a neoplasm has been detected and localized in a subject, a higher dose of labeled antibody, generally from 25 to 250 mCi for ^{131}I , and preferably from 50 nCi to 150 mCi per dose, based on a 70 kg patient weight, is injected. Injection may be intravenous, intraarterial, intralymphatic, intrathecal, or intracavitary, and may be repeated more than once. It may be advantageous for some therapies to administer multiple, divided doses of radiolabeled polypeptides or polypeptide mixtures, e.g., in the range of 20-120 mCi (70 kg patient), thus providing higher cell-killing doses to the neoplasm without usually effecting a proportional increase in radiation of normal tissues

Therapy using labeled polypeptides is advantageously used as a primary therapeutic treatment, but may also be used in combination with other anti-neoplastic therapies, e.g., radiation and chemotherapy, and as an adjunct to surgery. The

administration of such conjugated polypeptides is particularly useful in the case where small metastases cannot be surgically removed.

Combination of a Polypeptide with other Anti-Neoplastic Therapies

- 5 Chemotherapeutic agents and/or radiation and/or surgical removal of the neoplasm can optionally be combined with any of the methods of the present invention. Classes of compounds that can be used as the chemotherapeutic agent include: alkylating agents, antimetabolites, natural products and their derivatives, hormones and steroids (including synthetic analogs), and synthetics. Examples of
- 10 alkylating agents (e.g., nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes) include Uracil mustard, Chlormethine, Cyclophosphamide (Cytosan[®]), Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylene-melamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, and Temozolomide. Antimetabolites (including folic acid
- 15 antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors) may include, for example, Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, Pentostatine, and Gemcitabine. Natural products and their derivatives (including vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins) may also be
- 20 used and include, for example, Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, paclitaxel (paclitaxel is commercially available as Taxol, Mithramycin, Deoxyco-formycin, Mitomycin-C, L-Asparaginase, Interferons (especially IFN-alpha), Etoposide, and Teniposide. Hormones and steroids (including synthetic analogs) include, for
- 25 example, 17-alpha-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Tamoxifen, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, or Zoladex.
- 30 Exemplary synthetics (including inorganic complexes such as platinum coordination complexes) include Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine,

Mitotane, Mitoxantrone, Levamisole, and Hexamethylmelamine.

Methods and dosages for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the
5 'Physicians' Desk Reference' (PDR), e.g., 1996 edition (Medical Economics Company, Montvale, N.J. 07645-1742, USA), the disclosure of which is incorporated herein by reference.

The following examples are provided for the purpose of illustrating the
10 invention and should not be construed as limiting.

Example 1 Materials and Methods

15

Cell Culture

Human lung squamous cell carcinoma cell line LOU-NH91 was cultured in RPMI-1640 media (PAA, Vienna, Austria) supplemented with 20% fetal calf serum (FCS), 2mM glutamine and penicillin/streptomycin (both 1%) and incubated in a
20 humidified, 5% CO₂ atmosphere at 37°C. For the assays described, cells were grown to sub-confluency, detached with trypsin/EDTA and washed twice with phosphate-buffered saline (PBS) before use.

Producing Hybridomas

25 We immortalized lymphocytes by fusing them to the HAB-1 heteromyeloma as follows.

We washed the HAB-1 heteromyeloma cells twice with RPMI 1640 (PAA, Vienna, Austria) without additives and centrifuged the cells for 5 minutes at 1500 rpm. We then thawed frozen lymphocytes obtained from either the spleen or the
30 lymph nodes and we washed these cells twice with RPMI 1640 without additives and centrifuged these cells at 1500 rpm for 5 minutes. Both the HAB-1 and the lymphocyte cell pellets were resuspended in 10 ml RPMI 1640 without additives and

were counted in a Neubauer cell counting chamber. We washed the cells again, added the HAB-1 cells and the lymphocytes together in a ratio of 1:2 to 1:3, mixed them, and centrifuged the mixture for 8 minutes at 1500 rpm. We pre-warmed Polyethylene Glycol 1500 (PEG) to 37°C and carefully let the PEG run drop-wise onto the pellet
5 while slightly rotating the 50 ml tube. Next, we gently resuspended the pellet and rotated the tube for exactly 90 seconds in a 37°C water bath. We washed the cells twice with a full 10 ml pipette of RPMI without additives and centrifuged the cells for 5 minutes at 1500 rpm. We added 1 ml of RPMI 1640 with HAT supplement (PAA, Vienna, Austria) and 10% FCS, 1% glutamine, and 1% penicillin/streptomycin
10 ("RPMI 1640 HAT") into each well of a 24-well plate. The cell pellet was dissolved in RPMI 1640 HAT and 0.5 ml of the cells was added to each well of the 24-well plate. We then placed the 24-well plates into a 37°C incubator and changed the RPMI 1640 HAT medium weekly. After four to six weeks, the cell culture supernatants were screened for antibody production in an enzyme-linked immunosorbent assay
15 (ELISA).

Using this protocol, approximately 80% to 90% of the triomas generated are viable and approximately 50% secrete immunoglobulins. Positive clones were tested immunohistochemically on autologous tumor tissue sections and clones that showed a positive reaction were subsequently re-cloned.

20

cDNA Synthesis and RT-PCR

To obtain the sequence of the antibody, we isolated whole RNA from the trioma using the RNASE Kit from Qiagen. Total RNA may also be prepared using methods standard in the art, e.g., those described in Krenn et al. (Clin. Exp. Immunol.
25 115:168-175, 1999). cDNA synthesis from total RNA obtained from hybridoma cell line LM-1 (DSMZ Accession No. DSM ACC2623) was performed with 5 µg total RNA using Gibco BRL (Eggenstein, Germany) M-MLV Reverse Transcriptase according to the manufacturer's instructions. The amplification of V_H and V_L genes was carried out in a 25 µl volume with 1.75 mM MgCl₂, 0.4 pM primer, 200 µM of
30 each dNTP, and 1U Taq polymerase (MBI Fermentas, St. Leon-Rot, Germany). The PCR-products were amplified using the following cycle profiles: 95°C for 2 min,

followed by 35 cycles of 94°C for 30 sec; 65°C for 30 sec (for VH3 and VH4 primers), 60°C for VH1, VH2, VH5, VH6 and 52°C for VL primers respectively; a final extension at 72°C for 4 min.

5 *Sequencing the Antibody*

- The PCR products were purified using gel electrophoresis through 2% agarose (Roth, Karlsruhe, Germany) followed by gel extraction of the PCR product using a Jetsorb gel extraction kit (Genomed, Bad Oeynhausen, Germany). The PCR products were then cloned using the pCR-Script Amp SK⁺ cloning kit (Stratagene, Heidelberg, Germany). Ten positive clones were sequenced using the DyeDeoxy termination cycle sequencing kit (Applied BioSystems Inc., Weiterstadt, Germany) and analysed with an ABIPrism373 automated DNA sequencer (both strands were sequenced using T3 and T7 primers). The sequences were analysed using the DNASIS for Windows sequence comparison software and the GenBank and IMGT/V-QUEST databases.
- 15 The International Immunogenetics ("IMGT") database is coordinated by Marie-Paule Lefranc at the Université Montpellier, Montpellier, France.

Immunohistochemical Staining of Paraffin Sections

- Paraffin-embedded human tissues were sectioned (2µm), the paraffin was removed as follows:

- Two xylene washes for 5 minutes each,
- Two 100% ethanol washes for 5 minutes each,
- One methanol (70 ml) and H₂O₂ (500 µl) was for 5 minutes,
- Two 90% ethanol washes for 3 minutes each,
- 25 Two 80% ethanol washes for 3 minutes each,
- Two 70% ethanol washes for 3 minutes each, and
- washed in Tris/NaCl (3 grams Tris, 40.5 grams NaCl in 5 litres of distilled H₂O and pH adjusted to 7.4 with HCl).

- The slides containing the tissue sections were incubated in 300 ml distilled H₂O and citric acid (pH 5.5) in a pressure cooker at 100°C for 5 minutes. The slides were blocked for 15 minutes with 150µl of 0.5% Bovine Serum Albumin Fraction V

("BSA," Roth, Karlsruhe, Germany) in phosphate buffered saline ("PBS") per slide, and washed once with Tris/NaCl.

The sections were incubated with the primary antibody (e.g., LM-1, unrelated, human monoclonal IgM antibodies (ChromPure IgM, Dianova, Hamburg, Germany, 10µg/ml), CK8 antibody, or mouse CAM 5.2 antibody) diluted 1:50 (CAM 5.2 diluted 1:10) with BSA/PBS (Dako, Hamburg, Germany) for 2.5 hours in a humidified incubator at 37°C. The sections were then washed three times with Tris/NaCl (3 grams Tris, 40.5 grams NaCl in 5 litres of distilled H₂O and pH adjusted to 7.4 with HCl), followed by incubation with the secondary antibody (e.g., peroxidase-labeled rabbit anti-human or rabbit anti-mouse conjugate (Dako)) diluted 1:50 in PBS containing 30% rabbit serum at room temperature ("RT") for 1 hour. After washing three times with Tris/NaCl the tissue sections were incubated in PBS for 10 minutes before staining with 150µl diaminobenzidine (0.05%)-hydrogen peroxide (0.02%) for 10 minutes at RT. The reaction was stopped using running tap water (10-15 minutes) and the sections counterstained with hematoxylin. After mounting with glycerol-gelatin, the sections were analyzed using light microscopy.

Immunohistochemical Staining of Cryo-Sections from Autologous Tumors

Frozen human tissues were sectioned (4 µm) air-dried for two hours, fixed in acetone, air-dried for 30 minutes, and washed with Tris/NaCl (3 grams Tris, 40.5 grams NaCl in 5 litres of distilled H₂O and pH adjusted to 7.4 with HCl). The cryo-sections were then blocked with PBS containing 3% milk powder for 15-30 minutes at RT. After washing three times with Tris/NaCl the sections were incubated with LM-1 human IgM antibodies, unrelated human monoclonal IgM (Chrompure IgM, Dianova, 10µg/ml), CK8 (diluted 1:50 with BSA/PBS; Dako) or mouse CAM 5.2 antibody (diluted 1:10 with BSA/PBS) for 30 minutes at RT. The sections were washed three times with Tris/NaCl, followed by incubation with secondary antibodies (peroxidase-labeled rabbit anti-human or rabbit anti-mouse conjugate 1:50 in 70% PBS and 30% human serum) for 30 minutes at RT. After washing three times with Tris/NaCl and incubation in PBS for 10 minutes, the sections were stained with diaminobenzidine (0.05%)-hydrogen peroxide (0.02%) for 10 minutes at RT. The reaction was stopped

under running tap water and the sections counterstained with hematoxylin. After mounting with glycerol-gelatin, the sections were analyzed using light microscopy.

Preparation of tumor cell membrane extracts

- 5 Isolation of membrane proteins from tumor cells was performed as described using standard methods in the art, as described, for example, in Hensel et al. (Int. J. Cancer 81:229-235, 1999). In particular, confluent tumor cells (e.g., LOU-NH91 cells) were washed twice with PBS, harvested with a cell scraper, centrifuged, and resuspended in hypotonic buffer (20mM HEPES, 3mM KCl, 3mM MgCl₂) and
- 10 incubated for 15 minutes on ice. The cells were then sonicated for 5 minutes and the nuclei were pelleted by centrifugation at 10,000 x g for 10 min. The supernatant was centrifuged for 40 minutes at 100,000 x g in a swing-out rotor to pellet the membranes. After washing the pellet with hypotonic buffer, the pellet was resuspended in membrane lysis buffer (50 mM HEPES pH 7.4, 0.1 mM EDTA, 10%
- 15 glycerol, and 1% Triton X-100). Complete protease inhibitor (Boehringer, Mannheim, Germany) also was added to all solutions.

Western blotting

- Western blots were performed using standard techniques as described, for
- 20 example, in Hensel et al. (Int. J. Cancer 81:229-235, 1999). In short, blotted nitrocellulose membranes were blocked with PBS containing 3% low fat milk powder, followed by incubation for 1 hour with 20-40µg of LM-1 human IgM antibodies or unrelated human control IgM (ChromPure IgM, Dianova). The secondary antibody (peroxidase-coupled rabbit anti-human IgM antibody 1:1,000, Dianova) was detected
- 25 with the SUPERSIGNAL chemiluminescence kit from Pierce (KMF, St. Augustin, Germany).

Cytospin Preparation

- The adherent growing cells were detached by adding Trypsin/EDTA (PAA,
- 30 Vienna, Austria) followed by a 5 minute incubation in an humidified incubator (37°C, 5% CO₂) and centrifugation for 5 minutes at 1,500 rpm. The cells then were washed

twice with 10ml of RPMI-1640 cell culture medium (PAA, Vienna, Austria). The cell number was adjusted to a density of 1×10^5 cells/ml. From this solution, 100 μ l were centrifuged onto microscope slides with a cytopsin centrifuge (CYTOSPIN 2, Shandon, UK) for 2 minutes at 50 rpm. The resultant cytopsin were dried for at least 2 hours and stained as specified below.

Immunoperoxidase Staining of Cytopsin and Cryosections

Cytopsin were dried for at least two hours at room temperature or cryosections were dried for at least two hours after they were cut. The sections or cytopsin were then fixed for 10 minutes in acetone. The fixed cryosections/cytopsin were dried for 30 minutes at room temperature, washed three times with Tris-NaCl (3 grams Tris, 40.5 grams NaCl in 5 litres of distilled H₂O and pH adjusted to 7.4 with HCl), and placed into Tris/NaCl for 5 minutes. The cryosections/cytopsin were blocked for 15-30 minutes with 3% milk powder in PBS (100 μ l per cryosection/cytopsin) and washed three times with Tris-NaCl. The cryosections/cytopsin were incubated in 100 μ l of primary antibody per cryosection/cytopsin (e.g., at 20 μ g/ml in 0.5% BSA/PBS; CK 8 at 1:50 in BSA/PBS; CAM 5.2 at 1:10 in BSA/PBS; or RPMI 1640 media (PAA, Vienna, Austria) as a negative control) for 30 minutes in a humidified chamber at room temperature. Following the incubation, the cryosections/cytopsin were washed three times with Tris-NaCl.

The cryosections/cytopsin were then incubated in 100 μ l of a solution containing the secondary antibody (70 % PBS + 30% rabbit or human serum + e.g., 1:50 rabbit anti-mouse antibody, peroxidase coupled or 1:50 rabbit anti-human IgM antibody, peroxidase coupled; Dako, Hamburg, Germany) per cryosection/cytopsin for 30 minutes in a humidified chamber at room temperature and washed three times with Tris-NaCl and placed into PBS for 10 minutes. The cryosections/cytopsin were then incubated for 10 minutes in 100 μ l of a solution containing 0.05% diaminobenzidine and 0.02% hydrogen peroxide (Sigma, Taufkirchen (München), Germany). Following the incubation, the cryosections/cytopsin were washed with distilled H₂O and placed into a hematoxylin staining solution (Roth, Karlsruhe,

Germany) for 5 minutes. The cryosections/cytospins were then rinsed for 15 minutes under running tap water, washed with distilled H₂O, and cover with pre-warmed glycerol-gelatin.

The following experiments were carried out using the above materials and
5 methods.

Example 2

Generation of the Cell Line Expressing the LM-1

Monoclonal Antibody

10 As described above, we obtained the LM-1 monoclonal antibody expressing hybridoma by fusing lymphocytes obtained from the lymph nodes of a cancer patient with the heteromyeloma cell line HAB-1 (Faller, et al., Br. J. Cancer 62:595-598, 1990). The lymphoid sources were not pre-selected in terms of the age or sex of the patient. The resultant cell is a type of hybridoma known as a trioma, as it is the fusion
15 of three cells. Like normal B-lymphocytes, this trioma has to ability to produce antibodies. The specificity of the antibody is determined by the specificity of the original lymphocyte from the patient that was used to generate the trioma.

The hybridoma supernatants were screened for antibody production using an ELISA assay. Following ELISA, antibodies were primarily tested
20 immunohistochemically against their autologous tumor for tumor specific reactivity. The LM-1 antibody was generated from the lymphocytes of a patient with lung adenocarcinoma.

The amino acid sequence (SEQ ID NO:2) and the nucleic acid sequence (SEQ ID NO:1) of the variable region of the heavy chain of human monoclonal antibody
25 LM-1 are shown in Figure 7. As indicated in Figure 7, CDR1 of the LM-1 variable region heavy chain spans nucleotides 44-66 which encode amino acids 16-22, CDR2 spans nucleotides 109-156 which encode amino acids 37-52, and CDR3 spans nucleotides 253-309 which encode amino acids 85-103. In addition, the D region spans nucleotides 265-293 and the J region spans nucleotides 294-312.

30

Example 3

Immunohistochemical Characterization of an Antibody

To characterize the monoclonal antibody secreted by a hybridoma, we tested the antibody against a panel of normal and tumor tissues using an immunoperoxidase assay as described in the materials and methods. This assay provided us with an overview of which tissues were stained by the antibody and of the distribution of the antigen.

Antibodies that are specific for tumor cells and not for normal tissue were further characterized. First, we tested these antibodies against the same types of tumors from different patients. We then tested these antibodies against tumors of other organs and, finally, against normal tissues. Using these assays, we identified the human LM-1 monoclonal antibody. This tumor reactive antibody is of the IgM/ λ isotype (see Table 1).

Table 1: Origin of the LM-1 Monoclonal IgM Antibody and Clinical Data of Cancer Patients

Antibody	Organ	Tumour type	Tumour stage	Tumour grade	Age	Sex	Source of Lymphocytes	Ig Class
LM-1	Lung	Adenocarcinoma	T2N1	G3	45	M	Lymph Node	IgM/ λ

To investigate the genetic origin of this human monoclonal IgM antibodies the V_H gene was amplified, cloned and sequenced. The sequence was compared with germ-line sequences in the IMGT/V-QUEST database to identify the most homologous germ-line genes and to detect somatic mutations. The results are represented in Table 2. The degree of identity of the nucleotide sequences of the V_H segment to those of the closest reported germ-line V_H genes was approximately 99.6% as summarized in Table 2.

Table 2: Characterization of Variable Heavy Region of Monoclonal IgM Antibody LM-1

Antibody	Germ-line gene	Heavy chain		
		Homology (%)	R/S Frame	R/S CDR
LM-1	IGHV 4-30.01/ 4-31*01	99.6	1/0	0/0

5 Genes of the VH4 gene family expressed the LM-1 antibody. The high homology of the VH regions to the germ-line genes and the low R/S ratio, which is an indicator for affinity maturation of antibodies, indicates that none of the antibodies underwent affinity maturation by somatic mutation due to antigen contact. The data indicate that the LM-1 antibody belongs to the family of naturally occurring, non-
10 affinity matured antibodies.

After initial testing on autologous tumors, the reaction patterns of the antibodies were investigated in greater detail using immunohistochemical staining on a variety of paraffin- and cryo-embedded carcinomas and normal tissues. The LM-1 antibody exhibited no binding activity with normal tissues (Table 3).

15

Table 3: Reaction Pattern of the Monoclonal IgM Antibody LM-1 on Normal Tissues

Tissue	LM-1	CAM 5.2	M6 (IgM-Control)
Stomach	-	+	-
Colon	-	+	-
Lung	-	-	-
Esophagus	-	-	-
Urinary bladder	-	-	-
Prostate	-	-	-
Breast	-	-	-
Pancreas	-	-	-
Small Intestine	-	+	-

20 In contrast, the LM-1 antibody stains a variety of different tumor tissues (for details see Table 4).

Table 4: Reaction Pattern of the Monoclonal IgM Antibody LM-1 on Tumor Tissues

Tissue	Carcinoma type	LM-1 +/-	CAM5.2	M6 (IgM- Control)
Stomach	Adeno/diffuse	5/0	+	-
	Adeno/intestinal	2/1	+	-
Colon	Adeno	3/0	+	-
Lung	Adeno	5/1	+	-
	Squamous cell	6/0	+(CK5/6)	-
Esophagus	Squamous cell	3/0	+(CK5/6)	-
	Adeno (Barrett)	4/0	+	-
Pancreas	Adeno	6/0	+	-
Urinary bladder	Urothel	1/0	+	-
Kidney	Renal cell	1/0	-	-
Prostate	Adeno	7/0	+	-
Breast	Invasive (ductal)	4/0	+	-
	Invasive (lobular)	4/0	+	-
Ovary	Adeno	3/0	+	-
Uterus	Adeno	3/0	+	-

Antibody LM-1 gave a broad staining pattern on a variety of tumor tissues that were tested (Fig. 1). The positive control antibodies used in these experiments were a mouse monoclonal antibody against human cytokeratin 5/6 ("CK 5/6;" Dako A/S, Denmark) for squamous cell carcinoma of the lung and esophagus and a mouse monoclonal antibody against human cytokeratin ("CAM 5.2;" Becton Dickinson, New Jersey). Additional positive control antibodies (AE1/AE3 for adenocarcinoma of the colon and antibody CK8 for invasive ductal carcinoma of the breast) were used in the experiments shown in Figure 1.

To examine the antigen recognized by the LM-1 antibody, Western blots were performed with membrane extracts of established lung carcinoma cell line LOU-NH91. Antibody LM-1 reacted with an antigen with an approximate molecular weight of 70 kDa (Fig. 6). To rule out non-specific binding of IgM antibodies to membrane extracts, unrelated human control IgM was used as control.

Moreover, the LM-1 monoclonal antibody also specifically stains a number of carcinoma cell lines. In particular, the LM-1 antibody specifically binds lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383), and lung

squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393). Slides of these cells were stained according to the cytospin protocol described in the materials and methods section.

5

Example 4

Determining whether an Antibody Inhibits Cell Proliferation

Cell proliferation may be assayed by a number of methods that are standard in the art, for example, by the reduction of tetrazolium salts. The yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide ("MTT") (Sigma, St. Louis, MO), is reduced by metabolically active cells, in part by the action of mitochondrial dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The MTT cell proliferation assay measures the rate of cell proliferation and, when metabolic events lead to apoptosis, the reduction in cell viability.

For the MTT assay, we trypsinized cells (EPLC-272H or LOU-NH91) and resuspended the cells in 10 ml of RPMI-1460 medium containing 10% Fetal Calf Serum ("FCS") (20% FCS for LOU-NH91), 1% glutamine, and 1% penicillin/streptomycin (complete medium). The cells were then counted and diluted to 1×10^6 cells/ml. 50 μ l of this suspension were pipetted into wells of a 96-well plate, resulting in approximately 5×10^4 cells/well. The first row of wells was left empty. We then added 50 μ l of the antibody diluted in complete medium to each well. The 96-well plate was then incubated for 24 or 48 hours in a 37°C incubator. After the incubation period, 50 μ l MTT solution (5 mg/ml in PBS) were added to each well. The 96-well plate was incubated for 30 minutes at 37°C and centrifuged for 5 minutes at 800 x g. The supernatant was aspirated, 150 μ l of dimethylsulphoxide (DMSO) were added to each well, and the cell pellet was resuspended. Absorption was determined at a wavelength of 540 nm and at a reference wavelength of 690 nm in an ELISA reader.

As shown in Figure 2, after 24, monoclonal antibody LM-1 inhibited cell proliferation of lung carcinoma cell line LOU-NH91. In these experiments, LOU-

NH91 cells were incubated with the LM-1 monoclonal antibody, with depleted supernatant, or without an antibody for 24 hours. The y-axis shows the difference in absorbance at 540 nm and 690 nm ($A_{540}-A_{690}$). As is evident from these graphs, incubation with the LM-1 monoclonal antibody resulted in a decrease in cell proliferation and cell viability after both a 24 hour and a 48 hour incubation period.

Further exemplary results of such experiments are depicted in Figures 3A and 3B. After 24 or 48 hours, monoclonal antibody LM-1 inhibited cell proliferation of human epidermoid cell carcinoma cell line EPLC-272H in a concentration-dependent manner, while the controls with depleted cell culture supernatant remained unchanged (Figs. 3A and 3B).

Example 5

Determining whether an Antibody Induces Apoptosis

A number of assays standard in the art may be used to determine if an antibody induces apoptosis of a cell.

For example, we used the CELL DEATH DETECTION ELISA^{PLUS} (Roche, Mannheim, Germany) to analyze the extent to which the LM-1 antibody induce apoptosis. The cell death detection ELISA is based on a quantitative sandwich-enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones, respectively. This assay allows the specific determination of mono- and oligo-nucleosomes which are released into the cytoplasm of cells which die from apoptosis.

In particular, 1×10^4 tumor cells (LOU-NH91) were plated on 96-well plates and incubated in presence of different concentrations of the human IgM-antibody LM-1 for 24 hours at 37°C and 7% CO₂ in a CO₂ incubator. Depleted cell culture supernatant with unrelated IgM antibodies served as negative control. After the incubation period, the cells were centrifuged for 10 minutes at 200 x g and the supernatants were removed. The resulting cell pellets were then incubated with 200µl lysis-buffer for 30 minutes at room temperature. After centrifugation 20µl the supernatants were transferred into a streptavidin-coated microtiter plate (MTP) and 80µl immunoreagent (a mixture of 10% Anti-Histone-Biotin, 10% Anti-DNA-

peroxidase (Anti-DNA POD) and 80% incubation buffer) added before incubation for 2 hours at room temperature on a MTP shaker at 250 rpm. Following the incubation period, unbound components were removed by a washing step with incubation buffer. POD was determined photometrically with ABTSTM as a substrate (1 ABTSTM (2,2'-Azino-di[3-ethyl-benz-thiazolin-sufonate]) tablet in 5 ml substrate buffer). Antibody-induced apoptosis was measured by determining the color intensity of the green precipitate that it formed as a result of this reaction using an ELISA reader at a wavelength of 415nm in comparison to ABTSTM solution as a blank (reference wavelength of approximately 490 nm). Based on this color intensity, we calculated the level of the antibody-induced apoptosis. These experiments clearly showed that antibody LM-1 induces apoptosis in LOU-NH91 carcinoma cells after 24 hours of incubation (Figs. 4 and 5A) and after 48 hours of incubation (Fig. 5B). The Y-axis in these figures is the difference between the absorbance at 415 nm and at the 490 nm reference wavelength ($A_{415}-A_{490}$) and the medium control is RPMI 1460 medium. The concentration of the LM-1 antibody was either 25 μ g or 50 μ g/ml in supernatant.

Example 6

In Vivo Imaging of a Neoplasm

A patient suspected of having a neoplasm, such as a lung carcinoma, may be given a dose of radioiodinated LM-1 antibody, or another tumor-specific polypeptide, and radiolabeled unspecific antibody using the methods described herein. Localization of the tumor for imaging may be effected according to the procedure of Goldenberg et al. (N. Engl. J. Med., 298:1384, 1978). By I.V. an infusion of equal volumes of solutions of ¹³¹I-LM-1 antibody and Tc-99m-labeled unspecific antibody may be administered to a patient. Prior to administration of the reagents I.V., the patient is typically pre-tested for hypersensitivity to the antibody preparation (unlabeled) or to antibody of the same species as the antibody preparation. To block thyroid uptake of ¹³¹I, Lugol's solution is administered orally, beginning one or more days before injection of the radioiodinated antibody, at a dose of 5 drops twice or three-times daily. Images of various body regions and views may be taken at 4, 8, and 24 hours after injection of the labeled preparations. If present, the neoplasm, e.g., a

colorectal carcinoma, is detected by gamma camera imaging with subtraction of the Tc-99m counts from those of ^{131}I , as described for ^{131}I -labeled anti-CEA antibody and Tc-99m-labeled human serum albumin by DeLand et al. (Cancer Res. 40:3046, 1980). At 8 hours after injection, imaging is usually clear and improves with time up to the 24 hour scans.

Example 7

Treatment of a Neoplasm Using Labeled Antibody Mixtures

A patient diagnosed with a neoplasm, for example, a lung carcinoma, may be treated with the polypeptides of the invention as follows. Lugol's solution may be administered, e.g., 7 drops 3 times daily, to the patient. Subsequently, a therapeutic dose of ^{131}I -LM-1 antibody may be administered to the patient. For example, a ^{131}I dose of 50 mCi may be given weekly for 3 weeks, and then repeated at intervals adjusted on an individual basis, e.g., every three months, until hematological toxicity interrupts the therapy. The exact treatment regimen is generally determined by the attending physician or person supervising the treatment. The radioiodinated antibodies may be administered as slow I.V. infusions in 50 ml of sterile physiological saline. After the third injection dose, a reduction in the size of the primary tumor and metastases may be noted, particularly after the second therapy cycle, or 10 weeks after onset of therapy.

Example 8

Treatment Using Conjugated Antibodies

A patient diagnosed with a neoplasm, for example, a patient with a lung carcinoma that has metastasized to the chest, may be treated with solutions of ^{131}I -LM-1, ^{10}B -LM-1, and a Tc-99m labeled unspecific antibody. An amount of ^{131}I -labeled LM-1 antibody (in 50 ml of sterile physiological saline) sufficient to provide 100 mCi of ^{131}I activity based on a 70 kg patient weight may be administered to the patient. This dosage is equal to 3.3 mg of an antibody having 40-80 Boron atoms and 8-16 Boron-10 atoms per antibody molecule. The neoplasm is first precisely localized using the procedure of Example 6. In addition, Lugol's solution should be

continuously administered to the patient, as in the previous example. A well-collimated beam of thermal neutrons may then be focused on the defined tumor locations. Irradiation with an external neutron beam dose of 400-800 rads, delivered in a period of from 8-20 min, is effected for each tumor locus, and is optionally repeated with administration of the tumor-locating antibody, with or without the radiolabel, at intervals adjusted on an individual basis, but usually not exceeding a total dose of 3200 rads unless simultaneous external irradiation therapy is indicated. If desired, in addition to this therapy, an anti-tumor agent, such as a chemotherapeutic agent, may also be administered to the patient.

10

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

German Patent Application No. 103 11 248.0, U.S. Patent Nos. 5,367,060 and 5,641,869, and International Patent Publication Nos. WO 03/076472 and WO 2004/005351, and all other references cited herein are hereby incorporated by reference.

20

We claim:

25

Claims

1. A purified polypeptide that specifically binds to a neoplastic cell, but does not bind to a non-neoplastic cell, wherein said purified polypeptide specifically binds to a diffuse-type stomach adenocarcinoma, an intestinal-type stomach
5 adenocarcinoma, an adenocarcinoma of the colon, an adenocarcinoma of the lung, a squamous cell carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast, an
10 adenocarcinoma of the ovary, and an adenocarcinoma of the uterus, and not to non-neoplastic cells of the same tissue type, and wherein said purified polypeptide is substantially identical to the full-length sequence of SEQ ID NO:2.

2. The purified polypeptide of claim 1, wherein said polypeptide specifically
15 binds to at least one of lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383) or lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393).

20

3. The purified polypeptide of claim 1, wherein said polypeptide induces apoptosis of said neoplastic cell, but does not induce apoptosis of said non-neoplastic cell.

25

4. The purified polypeptide of claim 1, wherein said polypeptide decreases proliferation of said neoplastic cell, but does not decrease proliferation of said non-neoplastic cell.

5. A purified polypeptide comprising the amino acid sequence of SEQ ID
30 NO:2.

6. A purified polypeptide comprising amino acids 16-22, 37-52, and 85-103 of SEQ ID NO:2.

7. The purified polypeptide of claim 5 or 6, wherein said purified polypeptide specifically binds to a diffuse-type stomach adenocarcinoma, an intestinal-type stomach adenocarcinoma, an adenocarcinoma of the colon, an adenocarcinoma of the lung, a squamous cell carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast, an adenocarcinoma of the ovary, and an adenocarcinoma of the uterus, and not to non-neoplastic cells of the same tissue type.

8. The purified polypeptide of any one of claims 1-6 wherein said polypeptide is an antibody or a functional fragment thereof.

9. The purified polypeptide of claim 8, wherein said antibody is a monoclonal antibody or a functional fragment thereof.

10. The purified polypeptide of claim 8, wherein said functional fragment is selected from the group consisting of V_L , V_H , F_V , F_C , Fab, Fab', and $F(ab')_2$.

11. The purified polypeptide of any one of claims 1-6, wherein said polypeptide is also produced by the LM-1 cell line having DSMZ Deposit Accession No. DSM ACC2623.

12. A functional fragment of an antibody, wherein said functional fragment comprises amino acids 16-22, 37-52, or 85-103 of SEQ ID NO:2.

13. The functional fragment of claim 12, wherein said functional fragment is a functional fragment of a monoclonal antibody.

14. The functional fragment of claim 12, wherein said functional fragment is a V_H chain of an antibody.

5 15. A purified polypeptide that is specifically bound by an antibody, or functional fragment thereof, comprising the sequence of SEQ ID NO:2.

16. The purified polypeptide of claim 15, wherein said purified polypeptide is
10 a polypeptide expressed by lung adenocarcinoma cell line Colo-699 (DSMZ accession number ACC 196), lung adenocarcinoma cell line DV-90 (DSMZ accession number ACC 307), epidermoid lung carcinoma cell line EPLC-272H (DSMZ accession number ACC 383) or lung squamous cell carcinoma cell line LOU-NH91 (DSMZ accession number ACC 393), but not by non-neoplastic cells of the same tissue type.

15 17. The purified polypeptide of claim 15, wherein said polypeptide has an apparent molecular weight of approximately 70 kDa using sodium dodecyl sulfate polyacrylamide gel electrophoresis.

18. The purified polypeptide of claim 15, wherein said polypeptide, if present
20 on a cell and bound by said antibody or functional fragment thereof, results in apoptosis of said cell.

19. The purified polypeptide of claim 15, wherein said polypeptide, if present
25 on a cell and bound by said antibody or functional fragment thereof, results in a reduction in proliferation of said cell.

20. An isolated nucleic acid molecule comprising the sequence of SEQ ID NO:1.

30 21. An isolated nucleic acid molecule comprising nucleic acids 46-66, 109-156, and 253-309 of SEQ ID NO:1.

22. A vector comprising the nucleic acid sequence of claim 20 or 21.

23. An isolated cell comprising the vector of claim 22.

5

24. An isolated cell that expresses the polypeptide of any one of claims 1-10.

25. The isolated cell of claim 24, wherein said isolated cell is a mammalian cell.

10

26. The isolated cell of claim 25, wherein said mammalian cell is a human cell.

27. The isolated cell of claim 24, wherein said polypeptide is an antibody.

15

28. The isolated cell of claim 27, wherein said antibody is an IgM antibody.

29. The isolated cell of claim 24, wherein said antibody is a monoclonal antibody.

20

30. A method of producing the purified polypeptide of any one of claims 1-10, said method comprising contacting a cell with the vector of claim 22 and isolating the polypeptide expressed by said cell.

25 31. Use of the purified polypeptide of any one of claims 1-10 in a method of diagnosing a neoplasm in a mammal, said method comprising the steps of, (a) contacting a cell or tissue sample derived from said mammal with the purified polypeptide of any one of claims 1-10, and (b) detecting whether said purified polypeptide binds to said cell or tissue sample, wherein binding of said purified
30 polypeptide to said cell or tissue sample is indicative of said mammal having a neoplasm.

32. The use of claim 31, wherein said cell or tissue is derived from a stomach, a colon, a lung, an esophagus, a pancreas, a urinary bladder, a kidney, a prostate, a female breast, an ovary, or a uterus.

5

33. The use of claim 31, wherein said mammal is a human.

34. The use of claim 31, wherein said polypeptide is an antibody.

10

35. The use of claim 34, wherein said antibody is a monoclonal antibody.

36. The use of claim 31, wherein said polypeptide is conjugated to a detectable agent selected from the group consisting of a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, and a growth inhibitor.

15

37. The use of claim 36, wherein said detectable agent is capable of inducing apoptosis of said cell.

38. The use of claim 31, wherein said polypeptide is conjugated to a protein purification tag.

20

39. The use of claim 38, wherein said protein purification tag is cleavable.

40. Use of the purified polypeptide of any one of claims 1-10 in a method of treating a proliferative disorder in a mammal, said method comprising the step of contacting a cell with the purified polypeptide of any one of claims 1-10, wherein binding of said purified polypeptide to said cell results in a reduction in proliferation of said cell.

25

41. The use of claim 40, wherein said proliferative disorder is a diffuse-type stomach adenocarcinoma, an adenocarcinoma of the colon, an adenocarcinoma of the

30

lung, a squamous cell carcinoma of the lung, a squamous cell carcinoma of the esophagus, an adenocarcinoma of the pancreas, a urothel carcinoma of the urinary bladder, a renal cell carcinoma of the kidney, an adenocarcinoma of the prostate, an invasive ductal carcinoma of the breast, an invasive lobular carcinoma of the breast,
5 an adenocarcinoma of the ovary, or an adenocarcinoma of the uterus.

42. The use of claim 40, wherein said mammal is a human.

43. The use of claim 40, wherein said polypeptide is an antibody.

10

44. The use of claim 43, wherein said antibody is a monoclonal antibody.

45. The use of claim 40, wherein said polypeptide is conjugated to a detectable agent selected from the group consisting of a radionuclide, a fluorescent
15 marker, an enzyme, a cytotoxin, a cytokine, and a growth inhibitor.

46. The use of claim 45, wherein said detectable agent is capable of inhibiting cell proliferation of said cell.

20 47. The use of claim 40, wherein said polypeptide is conjugated to a protein purification tag.

48. The use of claim 47, wherein said protein purification tag is cleavable.

25 49. A medicament comprising the purified polypeptide of any one of claims 1-10 in a pharmaceutically acceptable carrier.

50. A diagnostic agent comprising the purified polypeptide of any one of claims 1-10.

30

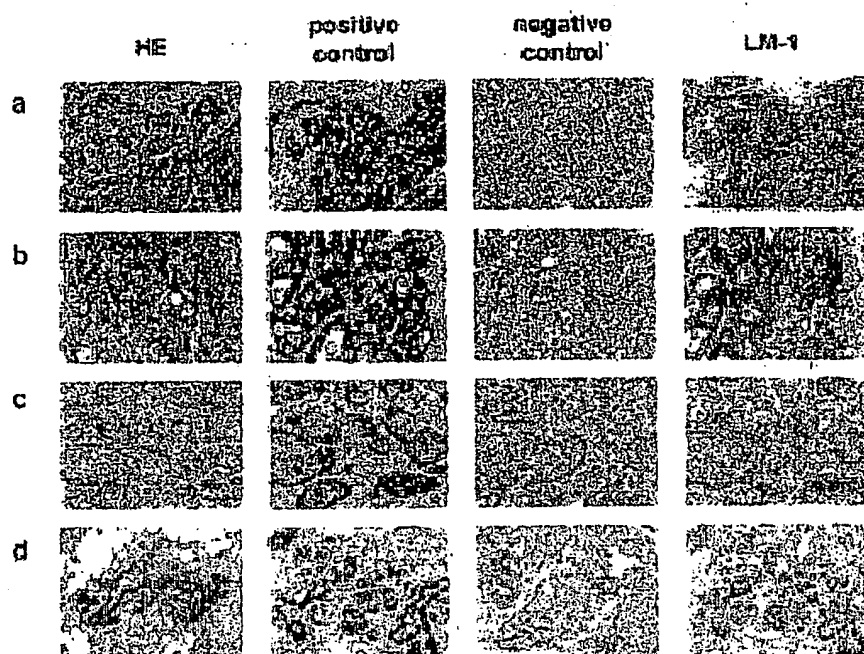


FIG. 1

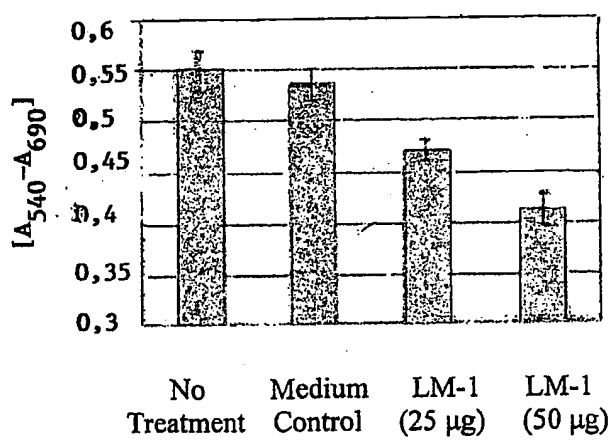


FIG. 2

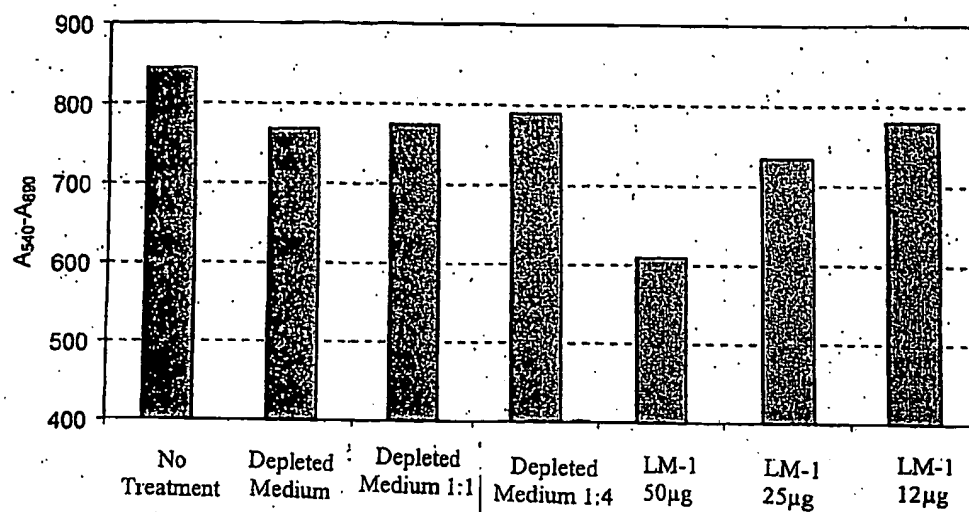


FIG. 3A

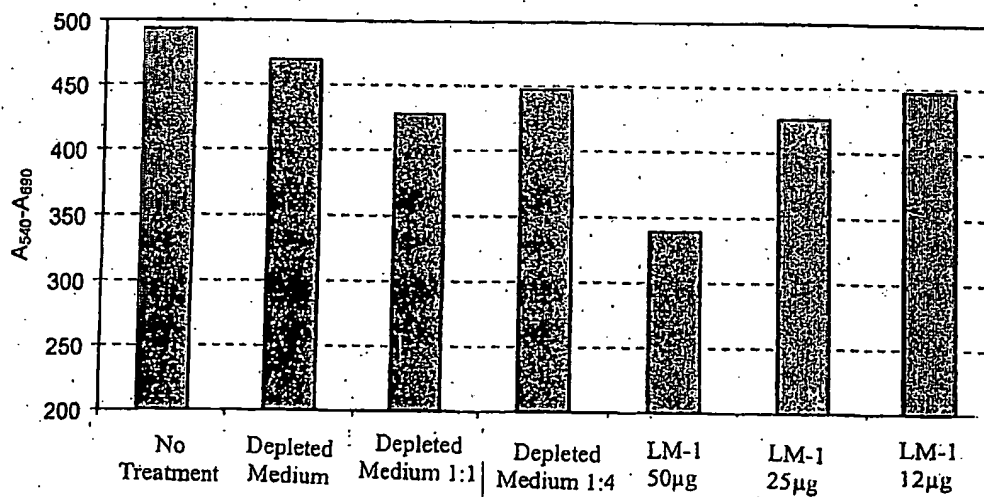


FIG. 3B

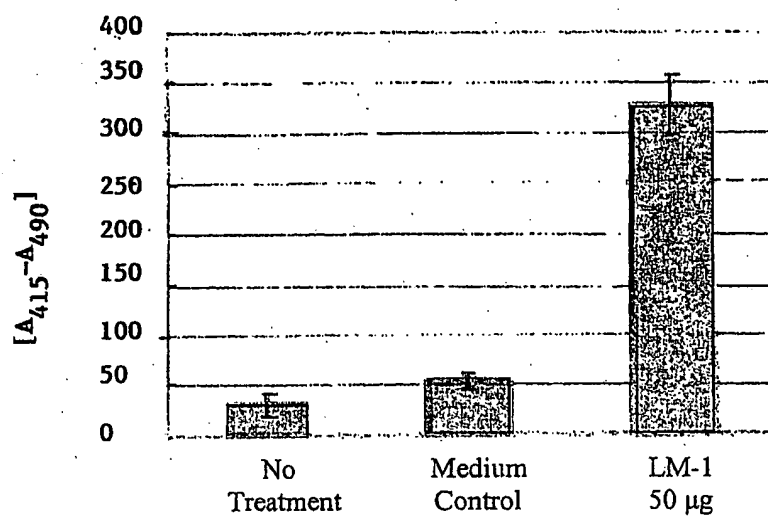


FIG. 4

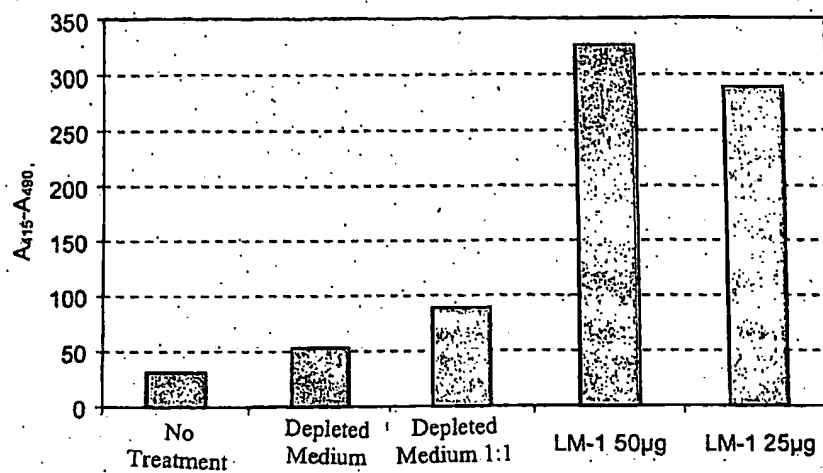


FIG. 5A

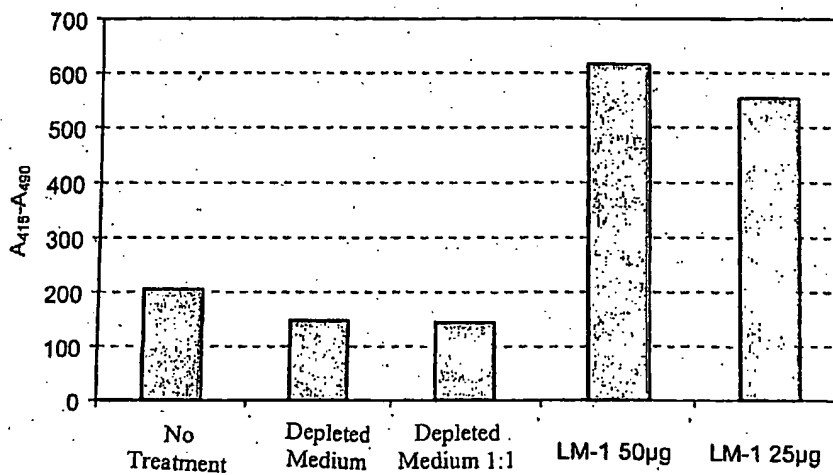


FIG. 5B

Control LM-1

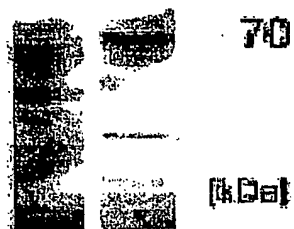


FIG. 6

CDR1		
ccg acc ctg tcc ctc acc tgc gct gtc tct ggt ggc tcc atc agc agt ggt ggt tac tac	60	
Pro Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Gly Gly Tyr Tyr	20	
1 5 10 15 20		
tgg agc tgg atc cgc cag cac cca ggg aag ggc ctg gag tgg att ggg tac atc tat tac	120	
Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Tyr	40	
25 30 35 40		
CDR2		
agt ggg agc acc tac tac aac ccg tcc ctc aag agt cga gtt acc ata tca gta gac acg	180	
Ser Gly Ser Thr Tyr Tyr Asn Pro Ser Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr	60	
45 50 55 60		
tct aag aac cag ttc tcc ctg aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat	240	
Ser Lys Asn Gln Phe Ser Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp thr Ala Val Tyr	80	
65 70 75 80		
CDR3		D-region
tac tgt gcg aga gtt gat gcg cga tat gat tac tct tgg ggg agt tat cgt tat gat gct	300	
Tyr Cys Ala Arg Val Asp Ala Arg Tyr Asp Tyr val Trp Gly Ser Tyr Arg Tyr Asp Ala	100	
85 90 95 100		
J-region		
ttt gat atc tgg ggc caa gga acc ctg gtc acc	330	
Phe Asp Ile Trp Gly Gln Gly Thr Leu Val Thr	110	
105 110		

FIG. 7

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.